HFE genotype and transferrin saturation in the United States

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Purpose: Examine the penetrance (defined by high transferrin saturation [TS]) of *C282Y* and *H63D* in the U.S. population. **Methods:** 5171 participants from the Third National Health and Nutrition Examination Survey, 1992 to 1994. **Results:** 77.1% (95% confidence interval [CI], 2.3, 95.1) of men and 51.9% (95% CI, 0, 84.2) of women with *C282Y* homozygosity had high TS. The associations of *H63D* homozygosity with high TS were stronger in people aged 50 years or older than in younger persons. Among Mexican-Americans, simple *H63D* heterozygosity was associated with high TS. **Conclusions:** The associations between *HFE* genotype and high TS may vary by sex, age, and ethnic group. ***Genet Med* 2003:5(4):304–310.**

Key Words: *hemochromatosis,* HFE *genotype, transferrin saturation, Mexican-Americans, non-Hispanic blacks*

In the United States, iron overload primarily results from hemochromatosis,[1] an autosomal recessive condition, characterized by lifelong excessive absorption of iron. Iron accumulates in body organs, eventually causing inflammation and damage. Cirrhosis of the liver, liver cancer, heart failure, diabetes, impotence, arthritis, and other disorders can result from hemochromatosis,[1–5] but early detection and treatment of this genetic condition can decrease morbidity and sometimes prevent the onset of disease.[2,3,5] On the basis of screening studies using iron indices, hemochromatosis is estimated to occur in 2 to 5 per 1000 persons in white populations in the USA.[6,7]

Although linkage of hemochromatosis to the major histocompatability complex (HLA) region on chromosome 6 has been recognized for more than 20 years,[8,9] the hemochromatosis (*HFE*) gene was first identified in 1996.[10] Two missense mutations in *HFE*, *C282Y* and *H63D*, account for most of the cases among whites. Homozygosity for *C282Y* accounts for 52 to 100% of these cases,[11] and a much smaller percentage of cases are attributed to *C282Y*/*H63D* compound heterozygosity and *H63D* homozygosity.[11] In the United States 0.26% (95% confidence intervals [CI], 0.12, 0.49) of persons are homozygous for the *C282Y* mutations, 2% are compound heterozygous (*C282Y*/*H63D*), and 2% are homozygous for the *H63D* mutation.[12] The prevalence of *C282Y* and *H63D* genotypes were similar for non-Hispanic whites.[12] Thus, a higher proportion of people at risk for hemochromatosis is revealed by *HFE* genotypes than by biochemical tests.

Limited clinical observations in screening studies suggest that an estimated 40% to70% of persons with the *C282Y* homozygous genotype develop clinical evidence of iron overload.[11] Simple heterozygosity for the *C282Y* or *H63D* mutation can be associated with increased levels of transferrin saturation (TS),[13–24] which is an initial indicator of hemochromatosis.[1] In case-control studies of whites, simple heterozygosity for the *C282Y* mutations was associated with a 4-fold increase in the odds of iron overload, and simple heterozygosity for the *H63D* mutation was associated with a 1.6-fold increase.[25] However, potential biases in selection and ascertainment of cases and controls, combined with a failure to consider factors such as age, race and ethnic group, sex, dietary factors, and alcohol consumption, may have decreased the accuracy of these estimates.[25] In addition, although iron overload increases with age and is more prevalent in males with hemochromatosis,[1] little is known about how age or sex affects the natural history of iron overload among persons with *H63D* or *C282Y* mutations. Finally, no study thus far has included racial and ethnic minorities in large enough groups to examine the association of *HFE* genotypes and transferrin saturation separately. The objective of the current study was to examine the association in the U.S. population by race and ethnic group, age, and sex.

MATERIALS AND METHODS

Survey design and participants

The National Health and Nutrition Examination Surveys (NHANES) are a series of national surveys that the National

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Center for Health Statistics, Centers for Disease Control and Prevention (CDC), began conducting in 1966 to estimate the prevalence and risk factors for common diseases in the U.S. population. NHANES III was conducted in two phases in 1988 to 1991 and 1992 to 1994.[26,27] Each phase was nationally representative, and it over-sampled certain populations including non-Hispanic blacks and Mexican-Americans. Population weights were calculated to account for oversampling, multistage sampling design, and nonresponse to the household interview and the examination.

Cell lines from 8,205 participants in NHANES III were immortalized with Epstein-Barr Virus (EBV) to establish a DNA bank. Although CDC planned to collect DNA for storage, the decision to establish cell lines occurred after phase 1 had already begun. Overall, 15,427 individuals were interviewed as part of phase 2. Of these, 10,280 were 12 years and older. A total of 10,052 individuals 12 years and older were examined, and cell lines were available for 7,195 (71.6%) of those examined. For the final analytic sample, participants were excluded if they were pregnant, were missing transferrin saturation, and were not Mexican-American, non-Hispanic white or non-Hispanic black, leaving 6,702 participants. To assure that previously anonymized specimens remained anonymous, special procedures were required by the Institutional Review Board (IRB) of the National Center for Health Statistics. Because of these procedures, 1,529 participants were eliminated randomly to decrease the ability to link specimens. Two additional specimens failed to amplify for genotyping, which left a final sample of 5,171.

Informed consent

The IRB approved the NHANES survey as well as this specific analysis. Although participants in the survey signed an informed consent form, specific mention of genetic research was not included. Because of the scientific importance of this resource, the NHANES IRB approved a process to make DNA available to the research community for anonymized testing. For this purpose, anonymized testing was defined as the inability for anyone, including CDC staff, to link the results of the genetic tests back to the survey participant.[28] Although participants were therefore not informed of their HFE genotype, they had been provided with iron metabolism laboratory test results during the course of the survey.

Transferrin saturation

Transferrin saturation (TS) was determined by dividing the concentration of serum iron (μmol/L) by total iron binding capacity (μmol/L) as assessed by a modification of the automated AAII-25 method that used ferrozine as the chromogen and dialysis to remove protein.[29] Both iron assays were conducted at the Central Laboratory for NHANES, National Center for Environmental Health, NCEH, CDC.

Genotyping methods

Specimens were genotyped in the Molecular Biology Branch of the Division of Laboratory Sciences, NCEH, CDC, using genomic DNA extracted from EBV-transformed cell lines. The wild type (designated as "+") and the *C282Y* and *H63D* mutations were genotyped using TaqMan technology,[30–33] in which amplification and genotyping are simultaneously performed using the ABI PRISM 7700 (Applied Biosystems, Foster City, California). These methods are described in detail elsewhere.[12]

Statistical analysis

As a result of the requirements to maintain anonymity, the analysis was broken into several steps. First, we used a weighted multiple linear regression with TS as the dependent variable and age, sex, race and ethnicity, fasting status, and time of day (and all interactions of age, sex, race, and ethnicity, up to and including three variables) as independent variables. The weights used were the NHANES III sampling weights for the examination in phase II. To test the validity of the model, we plotted the residuals against the predicted values and examined the distribution of residuals. We found that the variance did increase slightly with an increase in the predicted value, but transforming transferrin saturation using a square root or log function did not substantially improve the variance. The residuals were normally distributed with a mean of -0.10% and a standard deviation of 10.4%. The purpose of this preliminary analysis was to attempt to account for the variability in TS[34] (except that due to genetic factors) prior to linking race/ethnic group, sex, age group, mean weight, and decile of residual TS to genotype information. This analysis was done using the full NHANES III phase II database of 9041 persons who were 12 years and older, nonpregnant, with data on transferrin saturation, and who were non-Hispanic black, non-Hispanic white, or Mexican-American.

We focused our attention on the residuals from this model (i.e., the variability in TS levels that was unexplained by the variables in our model, which we denote "residual TS" or rTS). We ranked all persons by their residual TS value and categorized a person as having "high rTS" if their value of residual TS was in the highest decile. We next determined that our definition of high rTS corresponded well to high values of raw TS. The sensitivity of our high rTS variable for actual TS values $>$ 45% ranged from 98.8 to 100% across sex, race/ethnic, and age groups indicating that in most cases our threshold for high rTS was below 45%. Specificity ranged from 92 to 95.6% (Table 1). Sensitivity was lowest for white males, and specificity was lowest for non-Hispanic black females.

Sampling cells were created that contained no fewer than five persons who shared the same set of background characteristics (i.e., sex, race and ethnicity, age group, and decile of rTS). Of the persons within a sampling cell, 20% or 2 of the subjects, whichever was larger, were eliminated randomly. Thus the probability of selection of data from these cells varied from 60% (3/5) for cells $n = 5$ to 80% for cells $n = 8$ or greater. At this stage the link to individual identifiers was removed. The results of the genetic testing were then linked to the dataset using the random numbers that had been attached to both the DNA samples and the analytic dataset. Individual sample

Table 1
Sensitivity and specificity of high residual transferrin saturation[a] for transferrin saturation >45% in the US population, N = 9041[b]

Group	No. of subjects[c]	Sensitivity, %[d]	No. of subjects[e]	Specificity, %[f]
Male, non-Hispanic white	89	98.8	1362	95.6
Male, non-Hispanic black	69	100	1245	95.5
Male, Mexican-American	101	100	1220	95.3
Female, non-Hispanic white	82	100	1901	95.0
Female, non-Hispanic black	39	100	1631	92.0
Female, Mexican-American	29	100	1273	92.4
All sex and ethnic groups, age 12–49 y	300	99.7	5512	94.1
All sex and ethnic groups, age ≥50 y	109	100	3120	94.5

[a]Residual transferrin saturation in the 90th percentile or greater; residual TS is variability in raw TS levels unexplained by sex, racial and ethnic group, age group (and their interactions) fasting status, and time of day.
[b]Non-Hispanic black, non-Hispanic white, and Mexican American, non-pregnant subjects aged 12 years and older who participated in the Third National Health and Nutrition Examination Survey, 1992–1994, had a physical examination, and have values for transferrin saturation.
[c]No. of subjects who had transferrin saturation greater than 45% for a particular subgroup.
[d]Percentage of subjects who had high residual transferrin saturation, among those with a transferrin saturation greater than 45%.
[e]Number of subjects who had transferrin saturation less than or equal to 45% for a particular subgroup.
[f]Percent of subjects who had residual transferrin saturation less than the 90th percentile among those with a transferrin saturation less than or equal to 45%.

weights were replaced by average sample weights in each analytic cell. We adjusted the NHANES sample weights to account for the probability of selection of data from the cells given the number of people in each cell (see above). The final data set included the random number, age group, sex, race/ethnicity, residual TS decile, and adjusted sample weight.

We computed the weighted prevalence estimates of high rTS by genotype in the population.[27] Because we analyzed residuals that resulted after adjusting for age, race/ethnicity, and sex, we expect that the prevalence of high rTS is similar across these demographic variables. The associations between *HFE* genotypes and high rTS, as well as the interactions between each of the demographic variables (age, racial and ethnic group, and sex) and *HFE* genotype with respect to risk of high rTS, are separate and distinct from the associations between the demographic variables and high TS based on raw TS values.[35–37] Thus, the second step was to examine the interactions between *HFE* genotype and each demographic characteristic with respect to residual TS higher than the 90th percentile. Hence, we stratified our analysis of *HFE* genotype and high TS separately by each demographic characteristic (i.e., age, racial and ethnic group, and sex).

We used logistic regression to calculate the odds of high rTS by *HFE* genotype compared to that of the homozygous wild type in our final dataset as well as separately for each race/ethnic group, each age group (12–49, 50+) and each sex. As explained in Appendix 1[38] and above, it is still may be necessary to account for the interactions between demographic variables and the *HFE* mutations when computing the odds of high rTS even though values of rTS were calculated from a regression model that included these variables. The logistic regression was weighted using the sample weights described above to reproduce the odds of high rTS in the target (U.S.) population. For these analyses, the at-risk genotypes were *C282Y*/*C282Y*, *C282Y*/*H63D*, *H63D*/*H63D*, *C282Y*/+, and *H63D*/+, where + denotes the wild type. All logistic regressions were rescaled to the actual sample size using the NORMALIZED option after the weight statement in SAS.[39,40] To test potential interactions of HFE genotypes and demographic characteristics with the risk of high rTS, we calculated the difference in log likelihood ratios for a logistic regression model with the *HFE* genotypes and the specified demographic characteristic (e.g., age Group 50 or more years vs. age Group 12–49 years) compared with a model including the *HFE* genotypes, the demographic characteristic, and the interaction between the *HFE* genotypes and the demographic characteristic. We compared the difference in log-likelihood ratios with the tables for chi-square,[35] and we examined the significance of individual interaction terms. We corrected the odds ratios and 95% confidence intervals obtained from the logistic regression equations to better approximate prevalence ratios using the following equation:[41]

$$\text{Prevalence ratio} = \frac{\text{OR}}{(1 - \text{P}_0) + (\text{P}_0 \times \text{OR})}$$

OR equals the odds ratio, and P_0 is the prevalence of the outcome of interest (e.g., high rTS) in the +/+ group.

We were unable to calculate standard errors accounting for the complex sample design because anonymity requirements prevented access to cluster variables. Standard errors that do not account for the cluster variables would underestimate the true error of the estimates. Instead we used the binomial distribution[42] to construct approximate confidence intervals for the weighted prevalence estimates using an assumed design effect of 1.5, although in reality, the design effect for this analysis may be lower or higher than 1.5.[40] Random selection within strata tends to lower the design effect.[43] We also multiplied the errors from the coefficients of the logistic regression models by the square root of 1.5 in order to calculate confidence intervals corrected for the assumed design effect of 1.5. We used SAS, Version 8.1, for all analyses.[39]

RESULTS

Although the confidence intervals were wide, we observed that more persons with *C282Y* homozygosity had high rTS (69.1%, 95% CI, 17.3, 95.1) than persons without either mutation (8.8%, 95% CI, 7.8, 10.0) (Table 2). Persons with *H63D* homozygosity and compound heterozygosity were also more likely to have high rTS then persons without either mutation. The prevalence of high rTS appeared similar among males and females, but the number of persons with *C282Y* homozygosity

Table 2
Estimated prevalence of high rTS in the US population[a] by hemochromatosis (*HFE*) genotype and by sex

	Total		Males		Females	
HFE genotype	No. of subjects[b]	Weighted prevalence of high rTS, % (95% CI)[c]	No. of subjects[b]	Weighted prevalence of high rTS, % (95% CI)[c]	No. of subjects[b]	Weighted prevalence of high rTS, % (95% CI)[c]
C282Y/C282Y	8	69.1 (17.3, 95.1)[d]	5	77.1 (2.3, 91.7)[d]	3	51.9 (0, 84.2)[d]
C282Y/H63D	54	39.0 (25.5, 59.2)	23	31.8 (11.5, 60.6)	31	45.3 (26.2, 71.1)
H63D/H63D	67	25.1 (14.7, 42.2)	28	27.9 (12.8, 57.3)	39	22.6 (9.0, 43.6)
C282Y/+[e]	277	13.4 (9.0, 19.3)	103	14.6 (8.3, 26.9)	174	12.4 (7.4, 20.4)
H63D/+	887	9.9 (7.7, 12.7)	388	8.1 (5.1, 12.1)	499	11.6 (8.5, 15.7)
+/+	3878	8.9 (7.8, 10.0)	1740	9.0 (7.5, 10.9)	2138	8.7 (7.3, 10.3)

[a]Residual transferrin saturation in the 90th percentile or greater; rTS is the variability in raw TS values unexplained by sex, race/ethnic group, age group (or their interactions), fasting status, and time of day from the Third National Health and Nutrition Examination Survey, 1992–1994.
[b]Indicates the number of subjects who had positive test results for a particular genotype.
[c]Confidence intervals (CI) assume a design effect of 1.5.
[d]Estimate unstable due to small sample size.
[e]Absence of *C282Y* and *H63D* genotypes.

was too small to determine significant differences in the associations for any of the demographic subgroups.

The associations between high rTS and simple heterozygosity for *H63D* and *C282Y* in comparison with the wild type (+/+) were not statistically different by racial and ethnic groups (Table 3), except the association of simple *H63D* heterozygosity with high rTS was statistically significant only among Mexican-Americans. Among Mexican-Americans, those with simple *H63D* heterozygosity were 1.8 times (95% CI, 1.2, 2.6) as likely to have high rTS as those without either mutation (i.e., +/+). In comparison, non-Hispanic whites with simple *H63D* heterozygosity were 1.1 times (95% CI, 0.7, 1.6) as likely to have high rTS as those without either mutation.

Compared with persons aged 12 to 49 years with the +/+ genotype, persons aged 50 years and older with the same genotype were 0.6 times (95% CI, 0.4, 0.8) as likely to have high rTS (5.9% vs. 10.1%, see Table 4). Compound heterozygosity was associated with increased prevalence of high rTS in both age groups. The difference in the log-likelihood ratios for the models with and without the interactions with age group was statistically significant ($P < 0.025$) indicating a different association between *HFE* genotype in persons aged 50 years and older than in those < 50 years of age. Only the interactions for *H63D* homozygosity or simple *H63D* heterozygosity and age were statistically significant. The prevalence ratio for high rTS among persons with *H63D* homozygosity compared with the homozygous wild type (i.e., persons without either mutation) was 3.1 times (95% CI, 1.2, 9.9) greater among persons 50 years and older than the prevalence ratio among those < 50 years of age. The prevalence ratio for high rTS among persons with *H63D* heterozygosity compared with the homozygous wild type was 1.9 times (95% CI, 1.1, 8.8) greater among persons 50 years and older than the prevalence ratio among those < 50 years of age. The prevalence ratio among persons with *C282Y*

Table 3
Estimated prevalence of high rTS in the US population[a] by hemochromatosis (*HFE*) genotype and by race/ethnic group

	Non-Hispanic White		Non-Hispanic Black		Mexican-American	
HFE genotype	No. of subjects[b]	Weighted prevalence of high rTS, % (95% CI)[c]	No. of subjects[b]	Weighted prevalence of high rTS, % (95% CI)[c]	No. of subjects[b]	Weighted prevalence of high rTS, % (95% CI)[c]
C282Y/C282Y	6	67.9 (6.8, 93.2)[d]	1	100 (2.5, 100.0)[d]	1	100 (2.5, 100.0)[d]
C282Y/H63D	47	38.8 (24.3, 60.4)	1	100 (2.5, 100.0)[d]	6	31.9 (6.8, 93.2)[d]
H63D/H63D	48	24.4 (11.5, 43.4)	5	21.0 (8.3, 97.7)[d]	14	47.8 (20.3, 84.6)[d]
C282Y/+[e]	198	13.2 (8.3, 20.7)	38	15.6 (4.5, 35.7)	41	19.5 (8.5, 41.8)
H63D/+	477	9.3 (6.5, 13.2)	90	16.3 (8.3, 28.5)	320	15.7 (11.3, 21.6)
+/+	1240	8.7 (7.0, 11.0)	1465	9.5 (7.8, 11.6)	1173	8.7 (6.8, 10.9)

[a]Transferrin saturation in the 90th percentile or more; rTS is the variability in raw TS levels unexplained by sex, racial and ethnic group, age group (and their interactions), fasting status, and time of day from the Third National Health and Nutrition Examination Survey, 1992–1994.
[b]Indicates the number of subjects who had positive test results for a particular genotype.
[c]Confidence intervals (CI) assume a design effect of 1.5.
[d]Estimate unstable due to small sample size.
[e]+Absence of *C282Y* and *H63D* genotypes.

Table 4
Estimated prevalence of high rTS in the US population[a] by hemochromatosis (*HFE*) genotype and by age group, y

	12–49		≥50	
HFE genotype	No. of subjects[b]	Weighted prevalence of high rTS, % (95% CI)[c]	No. of subjects[b]	Weighted prevalence of high rTS, % (95% CI)[c]
C282Y/C282Y	3	73.1 (0, 84.2)[d]	5	63.7 (2.3, 91.7)[d]
C282Y/H63D	24	36.4 (15.2, 64.6)	30	43.7 (23.1, 68.5)
H63D/H63D	38	19.3 (6.7, 40.2)	29	35.2 (16.0, 60.8)
C282Y/+[e]	158	15.4 (9.7, 24.6)	119	9.7 (4.5, 18.9)
H63D/+	520	9.4 (6.6, 13.1)	367	10.8 (7.4, 15.6)
+/+	2683	10.1 (8.8, 11.6)	1195	5.9 (4.5, 7.9)

[a]Transferrin saturation in the 90th percentile or more; rTS is the variability in raw TS levels unexplained by sex, racial and ethnic group, age group (and their interactions), fasting status, and time of day from the Third National Health and Nutrition Examination Survey, 1992–1994.
[b]Indicates the number of subjects who had positive test results for a particular genotype.
[c]Confidence intervals (CI) assume a design effect of 1.5.
[d]Estimate unstable due to small sample size.
[e]+Absence of *C282Y* and *H63D* genotypes.

heterozygosity compared with the homozygous wild type was the same for persons 50 years and older and those < 50 years of age (prevalence ratio = 1.0, 95% CI, 0.5, 5.5).

DISCUSSION

We evaluated the association between TS, an indicator of circulating iron, and the *C282Y* and *H63D* mutations in the HFE gene in a representative sample of the U.S. population. Although the confidence intervals were wide, our estimate of about two-thirds of persons with *C282Y* homozygosity having high rTS is consistent with previous studies,[13–15,19–20,23,24] in which 50 to 100% of persons with *C282Y* homozygosity had high TS. Koziol and colleagues[13] reported that among 10,198 adults attending a health appraisal clinic in California, 48% of women and 81% of men with *C282Y* homozygosity (N = 42) had TS > 45%. Similarly, among whites in two different studies, 75[23] to 94[19] percent of persons with *C282Y* homozygosity had a TS > 45%. Distante and colleagues[15] studied a group of 505 unrelated hospital employees and found that both of the two employees with *C282Y* homozygosity had a TS > 50%. Deugnier and colleagues[44] found among people attending a health appraisal clinic in France that 41% of women ($n = 44$) and 80% of men ($n = 10$) with *C282Y* homozygosity had elevated TS compared with 5% of nonhomozygotes. Phatak et al.[24] found among 4,865 unselected primary care patients that 75% of men ($n = 4$) and 100% of women ($n = 8$) had a TS >45%. Although the majority of male *C282Y* homozygotes have elevated transferrin saturation in our study and most other studies to date, in the larger studies, including ours, usually only about half of the women do. The exception is the study conducted among primary care patients where 8 of out 8 women with C282Y homozygosity had high TS levels.[24] In Phatak's study,[24] the mean age of the patients was 52 years. The lower proportion of high TS levels among females than males in the majority of studies may be explained by loss of iron due to menstruation and pregnancy among women of childbearing age.

Our study had the largest sample of Mexican Americans and blacks thus far with *HFE* genotype and TS values. Our data suggested that simple heterozygosity for *H63D* was significantly associated with elevated rTS in Mexican-Americans. However, the lower prevalence of *C282Y* mutations in Mexican-Americans and non-Hispanic blacks,[12] and the resulting small sample size of exposed persons, make it difficult to address the associations between *C282Y* mutations and high rTS in these groups. Because of the low prevalence of *C282Y* and the strong association between *H63D* and high rTS in Mexican-Americans, the attributable fraction of clinical hemochromatosis arising from *H63D* mutations may be higher than that arising from *C282Y* mutations in this group. This possibility requires further investigation in screening studies that use genetic and iron testing for hemochromatosis among larger samples of Mexican-Americans.

Our study indicates that the associations between the *H63D* homozygosity and high rTS strengthen with increasing age. In screening studies for hemochromatosis using elevated serum iron measures and HLA-typing, symptoms and disease complications increased with age among persons considered homozygous for hemochromatosis.[1] The small sample of people with *C282Y* homozygosity and compound heterozygosity decreased our ability to examine these groups. The results by age are unclear for simple *H63D* or *C282Y* heterozygosity. The association with high rTS was slightly greater among older persons with simple *H63D* heterozygosity, but not among those with simple *C282Y* heterozygosity. The finding was consistent with a previous study[20] that found no difference in the association between simple *C282Y* heterozygosity and serum ferritin distribution by age.

The small number of variables and the inability to use continuous outcomes limited our analysis. We could not use the common thresholds for TS (e.g., 45%–70%) because sample

cell sizes became too small in some subgroups to meet the requirements for protection of the identity of human subjects. There is no consensus about the optimal TS threshold to identify people for further evaluation for hemochromatosis. Our TS threshold ($>$ 90th percentile of rTS) needed to maintain anonymity was slightly $<$ 45% in some population sub groups, but had comparable sensitivity and specificity to a threshold of 45%, the low end of phenotypic expression of hemochromatosis.[45] Because 45% is at the lower end of phenotypic expression, it generally has a high sensitivity, but low specificity for diagnosis of hemochromatosis.[45] Similarly, because the sample size would become too small in some subgroups to preserve anonymity, we could not include many variables that may affect transferrin saturation values such as anemia of various causes, inflammation, megaloblastic conditions, or hepatocellular damage attributable to concurrent viral disease, alcohol, or drugs. Thus we cannot rule out that high TS was due to these factors. The sample size limited our ability to precisely estimate the association of *C282Y* homozygosity with high TS and the associations of *H63D* homozygosity and compound heterozygosity with high TS for some of the demographic subgroups. Finally, we only had one measure of TS, and although we were able to adjust for fasting status and time of day of specimen collection, because of the biological variability in TS, repeated measures are needed to better establish risk of hemochromatosis.[1,45]

The results of our study and others together indicate that although genotype and TS are correlated, a substantial proportion of people who are homozygous for *C282Y*, and the majority of people who are heterozygous for *HFE* genotypes, do not have high TS. In addition, our study suggests that the association between *HFE* genotype and high TS may vary with age. In Mexican-Americans, the presence of *H63D* may be more important than *C282Y* as a risk factor for hemochromatosis because of its higher prevalence and its positive association with transferrin saturation. Our study reaffirms the need for additional information about the risk of iron overload and chronic disease associated with *HFE* genotypes, as well as other genetic and environmental factors that modify this risk, to make informed decisions regarding genetic screening for hemochromatosis. Much of this information will be gained through the National Institutes of Health sponsored Hemochromatosis and Iron Overload Screening (HEIRS) Study, a multicenter screening study including 10,000 Hispanic and 27,000 African American individuals.[46] Because iron overload has a variable age of onset, may be influenced by dietary, alcohol, and other factors, and may be affected by modifier genes, a longitudinal study is required to determine the true penetrance of the *HFE* genotypes.

APPENDIX

In this appendix, we show that inference about levels of high TS (as defined in the statistical methods section) obtained from a logistic regression is directly related to inference about the effect of genotype on raw TS levels.

We assume TS levels in the target population follow a linear model with a vector of demographic effects x, a genotype effect g and a vector of gene by demographic effect interaction gx. Here, x may include both main effects of individual demographic variables and interactions between demographic variables. We use the term demographic loosely to include variables like fasting status and time of day. Hence, the model underlying our analyses is as follows:

$$TS = a_1 x + a_2 g + a_3 gx + \epsilon \qquad (1)$$

where ϵ is the error in this model that we assume is normally distributed with mean 0 and variance σ^2. We assume one component of x is the variable 1 so that a separate intercept is not necessary.

In the first stage of our analysis, we fit a model for TS levels as a function of demographic variables x only, namely, we fit the model:

$$TS = a_1' x + \epsilon' \qquad (2)$$

where the coefficients a'_1 and error ϵ' do not necessarily equal coefficients a_1 and error ϵ in the underlying model unless g and gx are orthogonal to x. Having fit Equation 2 and obtained estimated coefficients $\hat{a}'_1$, we considered residuals TS values defined by $R = TS - \hat{a}'_1 x$.

However, using Equation 1 we see that R satisfies

$$R = \alpha x + a_2 g + a_3 gx + \epsilon \qquad (3)$$

where $\alpha = a_1 - \hat{a}'_1$. Note that α is not equal to 0 because the coefficients a'_1 are not equal to a_1 in general.

Given model Equation 3, we can calculate the probability that residual TS is greater than some cutoff value c. Conditional on the value of $\hat{a}'_1$ in the first stage analysis, the error in Equation 3 is normally distributed with mean 0 and variance σ^2. Hence, we find the following:

$$Pr[R > c | x, g, \hat{a}'_1] = \Phi\left(\frac{c}{\sigma} - \frac{\alpha}{\sigma}x - \frac{a_2}{\sigma}g - \frac{a_3}{\sigma}gx\right) \qquad (4)$$

Equation 4 shows that the occurrence of high TS follows a probit regression model in the population, with regression coefficients for g and gx that are proportional to their analogues in Equation 1. Because the logistic function gives a very close approximation to the cumulative distribution of the normal distribution, logistic regression approximates probit regression (see e.g., Ashford et al.[47] and references therein). Hence, the coefficients of genotype g and the interactions between genotype and demographic variables gx in a logistic regression for factors that predict high (residual) TS are proportional to the analogous coefficients in the original linear model Equation 1 for raw TS.

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